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ORIGINAL CONTRIBUTION

Multinational study of major breast milk carotenoids of healthy mothers

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Summary *Background* Carotenoids in serum vary between countries and within populations with evidence suggesting a qualitative relationship to diet. Breast milk carotenoids furnish a source of vitamin A and potentially provide immunoprotection and other health benefits for infants. There have been numerous studies of milk carotenoid concentrations in undernourished populations; however, carotenoid concentrations have not previously been compared in populations of well-nourished mothers. *Aim of Study* To compare concentrations of five major carotenoid groups: α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene in breast milk of healthy women from Australia, Canada, Chile, China, Japan, Mexico, the Philippines, the United Kingdom, and the United States, and to qualitatively compare patterns of dietary intake with milk carotenoid concentrations. *Method*s Breast milk collected from healthy lactating women was analyzed for concentrations of five carotenoids and retinol and quantitated relative to total milk lipid. All determinations were performed in a single research laboratory using standardized methodology. Mothers consumed their usual diets and provided a single 24-h dietary recall. *Results* Breast milk carotenoid concentrations varied greatly among countries, with the greatest differences in β-cryptoxanthin (~ 9-fold) and the least in α-carotene and lycopene (~3-fold). Breast milk retinol concentrations varied ~ 2-fold across countries. The provitamin A carotenoids α-carotene, β-carotene, and β-cryptoxanthin as a group accounted for > 50 % of the carotenoids measured. Total breast milk carotenoids were highest in Japanese and lowest in Philippine mothers. Breast milk β-carotene concentrations were highest in Chile and lowest in the Philippines. *Conclusions* Patterns of breast milk carotenoids were unique to each country and qualitative patterns reflected the dietary carotenoid supply.

Key words breast milk – carotenoids – healthy mothers

EJN 403

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Introduction

As constituents of breast milk, the provitamin A carotenoids (β-carotene, α-carotene and β-cryptoxanthin) provide a significant source of vitamin A for the nursing infant. Furthermore, 30 additional carotenoids identified in breast milk have no known provitamin A activity but many have been associated with health benefits. For example, lycopene, a powerful antioxidant, is associated with decreased risk for ovarian [1] and prostate cancer [2], while the macular pigments lutein and zeaxanthin are associated with decreased risk for age-related macular degeneration and cataracts [3]. Carotenoids as a group may enhance the immune system [4] and provide protection against chronic diseases [5]. Carotenoids delivered in breast milk may enhance immune defenses of the infant and protect against chronic disease later in life.

Breast milk is the preferred source of nutrition for infants. In addition to providing nutrients for growth, it contains biological components such as antibodies, enzymes and hormones, all of which contribute to infant development and protection of health. However, breast-feeding is not always possible. When artificial feedings are required, the nutrient composition of the infant formula should reflect the nutrient composition of breast milk as closely as possible. Unfortunately, many infant formulas currently on the market contain no measurable levels of carotenoids, and those that do, contain predominately β-carotene and β-cryptoxanthin [6].

Previously, most studies of carotenoids in breast milk have been of healthy U. S. mothers or mothers consuming diets low in vitamin A [7–12]. Milk carotenoid concentrations of healthy mothers in other countries have not been compared with those of U. S. mothers and in early studies, carotenoid concentrations in breast milk from U. S. mothers were assumed to be the standard "normal" concentrations [13]. If breast milk carotenoid composition did not vary between populations it would then be appropriate to use U. S. data as a reference against which to compare breast milk carotenoids in vitamin A low-to-deficient populations [14–18], and for the design of infant formula. However, if patterns of breast milk carotenoids within a population reflect the carotenoids in the local food supply, data from a single country would not provide a worldwide reference for healthy mothers. Therefore, to assess the range of carotenoid intakes for healthy breast-fed infants worldwide, comparative baseline data are needed. For this study, we chose nine countries that varied in climate and cultures and in which we were able to identify a pediatrician who had research experience and was involved with a successful lactation program. Five of the countries selected were in Asia or the Pacific Rim, three were in the Americas and one was in Europe (UK).

Subjects and methods

Study design

The study was a single, cross-sectional survey of carotenoids in milk from apparently healthy, well-nourished lactating women in nine countries: Australia, Canada, Chile, China, Japan, Mexico, the Philippines, the United Kingdom, and the United States. Study sites/investigators were selected using the following criteria: 1) participation by a practicing pediatrician, 2) clinic, health center or hospital-based, 3) access to a large group of healthy, lactating women, 4) available staff experienced in collection of breast milk, and 5) adequate facilities for the collection and storage of breast milk.

All mothers signed written, informed consent in their native language prior to enrolment in the study.

Subject Selection

All participants were 18 to 40 years of age, mothers of a healthy full-term singleton infant, and between 1–12 months postpartum at the time of milk collection. The sample collection period at each site was limited to three months. Our goal was to obtain samples from 50 women in each country. Inclusion criteria required: 1) a minimum 8.5 kg prenatal weight gain to assure that women had not followed a restrictive diet during pregnancy, 2) frequency of breast-feeding at least 5 times per day to assure that infants were primarily breast-fed, 3) consumption of at least 3 servings of fruits and vegetables (combined) per day, and 4) ability to comply with an afternoon appointment time. Exclusion criteria disallowed mothers who smoked, were receiving steroid medications, taking vitamins or other supplements containing carotenoids or vitamin A > 8000 IU/day, or who had given birth to more than 5 infants. The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Human Subjects Committee of the University of Arizona and the Human Ethics Committees associated with each participating institution.

Prior to the study, a clinical scientist (KP) and/or the research investigator (LC) travelled to each site to train nursing personnel on the proper use of the electric breast pump, using a videotape created for that purpose. The study procedure for recruitment/enrolment, milk collection, sample handling and dietary interviewing were also discussed. Mothers were recruited during an appointment with their pediatrician, signed Informed Consent forms, and made an appointment to return mid-afternoon on the following day to express their milk. On the day of sample collection, nurses interviewed mothers for a health history for their infants and themselves and completed a 24-h dietary recall with

them to record foods consumed during the previous day. Mothers were asked to report serving sizes in volumes, number of pieces or weight. Nurses were instructed to prompt the mothers for additional details as needed.

Dietary records

An attempt was made to schedule appointments for participants during the season of the year when fresh fruits and vegetables were most plentiful at the various sites; however on-site investigators reported minimal seasonal variation of fruits and vegetables in their geographic locations and the effort was abandoned. Study nurses reviewed mothers' 24-hour dietary intake records at the appointment for milk collection. A registered dietician calculated the number of servings of the fruit or vegetable from each diet record using the American Dietetic Association Exchange Lists. An international carotenoid database that included all the fruits and vegetables recorded by our participants was not available and reported carotenoid contents for foods varied significantly among the various databases that were available. Therefore, we elected to rank intake of fruits and vegetables containing carotenoids rather than to attempt to quantitate individual carotenoid intake. For each country, the number of servings of each fruit and vegetable were tallied (Microsoft Excel 2000, Microsoft Corp). Items with the highest counts were listed as those most frequently consumed in that country. Quantities of fruit and vegetable servings were verified by duplicate entry by two data analysts.

Collection of Samples

Data previously reported by one of us (LC) provides evidence that a single, mid-afternoon sample of breast milk adequately represents the average 24-h concentration for each of the five carotenoids [7]. Therefore, a single, complete breast expression was collected between 1300 and 1700 hours from each mother using an electric pump. Because milk lipid concentrations vary significantly throughout the duration of a feed [7, 19], with foremilk being low in lipid content relative to hindmilk, a full breast expression collected by electric breast pump was the preferred method for collection [7, 8]. However, in Japan where the use of an electric pump is unacceptable to women, mothers expressed their milk manually using a hand-held breast pump under supervision of clinic staff. To control for differences in collection methods, all milk carotenoid data were reported relative to lipid content. For consistency, and to avoid obtaining residual hind milk, samples were collected from the breast from which the infant had most recently fed. When both breasts had been used during the previous feeding, the sample was collected from the breast that felt the most "full" by the mother's report. Because retinol and carotenoid concentrations are higher in colostrum than in mature milk [20], and because milk retinol and carotenoid concentrations are most stable during months 1–12 of lactation [13] we excluded women who had breast-fed for less than 1 month or more than one year.

Subject remuneration

Mothers were reimbursed for travel expenses and, when allowed by local ethics committees, a token baby gift was provided to the mother. In addition, information on the carotenoid composition of their breast milk was made available to mothers at study completion.

Sample handling

Carotenoids are very sensitive to both light and oxygen, therefore precautions were taken to minimize exposure of breast milk samples to air and/or light throughout all phases of the study. Samples were collected in sterile, opaque polypropylene collection bottles labeled with the infant's birthdate, date of milk collection, and initials of the mother. Aluminum foil covered the entire bottle and a computer scan code was placed on the outside of the foil for identification of the specimen. Expression of milk from one breast was continued until milk flow ceased. Samples were then placed in a self-sealing plastic bag along with a completed requisition sheet, from which data were entered into a database by the certified laboratory. If two collection bottles were required because of the volume of milk, both bottles were placed together in one bag. Bags containing samples were placed immediately on dry ice or in a freezer at –20 °C. Samples containing less than 60 mL were considered an incomplete expression and were not included in data analysis. After 10 samples were collected at a given site, a shipment was arranged. Frozen samples were packed on dry ice in styrofoam shipping containers provided by World Courier, who also provided dry ice replenishment enroute to an accredited laboratory[1]. Sample labels placed over the aluminum foil were scanned into an inventory system and samples were stored at –70 °C. Data from requisition slips accompanying samples were entered into a database corresponding to the electronic code for each sample. The sponsor was notified of sample details at each delivery. When the laboratory was informed by

1 In August 1999, Quest Diagnostics, Inc acquired Smith Kline Beecham Clinical Laboratories, Inc from Smith Kline Beecham Corporation. The clinical trials business is now known as Quest Diagnostics Clinical Trials.

the sponsor that all samples from a given country had been received, the following sampling procedure was performed by the assigned laboratory technicians. Frozen samples from a single country were thawed overnight in the refrigerator. In a room illuminated by subdued (yellow) light, thawed samples were removed from the refrigerator and immersed in a warm water bath until the temperature reached 37 °C. Samples were then gently stirred using a magnetic stirring bar for 10 minutes. A 10 mL portion of each breast milk sample was taken by pipette from the vortex and placed in clean, sterile polypropylene bottles. These bottles were labeled with duplicate copies of the original sample labels and returned to the –70 °C freezer until they were sent as a group, on dry ice, to the analytical laboratory in Tucson, AZ. The remaining milk samples were returned to the –70°C freezer until shipment to the research laboratory of one of us (JJ) for future analyses.

Analysis of milk retinol and carotenoids

A master pool of mature breast milk was constructed by combining single breast expressions (50–200 mL) from seven healthy donors in the Tucson metropolitan area. When analysis of the field samples began, samples for batch analysis were prepared from the master pool as previously described [14]. The master milk pool was thawed at 37 °C in an orbital shaker (Model 35127, Labline, Inc, Melrose Park, IL, 130 oscillation/min, 10 min) and multiple 1 mL portions removed and stored in darkened polycarbonate vials at –70 °C. A single pool sample was thawed and analyzed with each batch of experimental samples. Samples from the same master pool were used throughout the study.

Extraction and HPLC analysis of carotenoids were performed as previously described [9]. All procedures were performed under subdued lighting at 25 °C. Because our HPLC system did not resolve them, concentrations of the xanthophylls lutein and zeaxanthin are reported together. The limit of detection for milk carotenoids using our HPLC method was 0.0025 µmol/L.

Quantitation of milk lipids

Milk lipid concentrations were determined by "creamatocrit" as previously described [21] and verified by standard gravimetric methods. Data are the average of three determinations from a single sample.

Materials

All chemicals were technical grade or better and were obtained from Aldrich (Milwaukee, WI) or Sigma Chemical Co. (St. Louis, MO). Solvents for chromatography were HPLC grade from Burdick Jackson (Muskegon, MI). Ethanol was obtained from Quantam Chemical Corp., USI Division (Tuscola, IL).

Data analysis

Parity for individual countries is expressed as a median. All other data (carotenoids, retinol, lipids, total carotenoids and maternal and infant ages) are expressed as mean ± SEM. Differences among the nine countries were analyzed for each carotenoid by covariance analysis using age as the covariate. Dunnett's Test was applied to the means using the U. S. data as a standard to determine differences from the U. S. site mean. Differences were considered statistically significant at $p \leq 0.05$.

Results

Anthropometrics

The mean maternal age for all countries was 29.6 yr (Table 1). There were significant differences in age among the nine countries with two distinct groups evident following multiple range test analysis of the site means. Mothers in Chile, the Philippines, and China were younger (mean ages 26.3, 26.9, and 27.5 yr respectively) compared to the remaining six countries (mean ages 30.1–32.1 yr). However, within these two groups age differences were not significant.

Age and parity were strongly correlated (Pearson Correlation Coefficient = 0.36 for 465 paired age and parity observations, $p < 0.0001$). Therefore, analysis of only one variable was needed to assess the influence of either age or parity on the magnitude of the carotenoid measurements. Age was chosen as the covariate for these analyses. There was no significant effect of age, and therefore parity, on the variability of any of the individ-

Table 1 Subject characteristics

Country	Mother's age (years ± SEM)	Infant age (days ± SEM)	Parity (median)	n
Australia	30.1 ± 0.6	121 ± 7	2.0	51
Canada	32.1 ± 0.5	129 ± 7	2.0	55
Chile	26.3 ± 0.9	87 ± 12	2.0	49
China	27.5 ± 0.4	74 ± 5	1.0	52
Japan	31.7 ± 0.4	98 ± 7	1.0	50
Mexico	30.3 ± 0.6	124 ± 9	1.5	50
Philippines	26.9 ± 0.7	67 ± 4	2.0	60
United Kingdom	31.7 ± 0.7	74 ± 7	1.5	50
United States	30.2 ± 0.7	116 ± 11	1.0	48

ual milk carotenoids, total carotenoids, or retinol concentrations.

Dietary analysis

Since fruits and vegetables were considered to be the only significant sources of carotenoids, these were the foods whose consumption we reviewed most carefully. We did not calculate carotenoid composition of foods since a single 24-h food record is not a reliable predictor of carotenoid intake from foods [22]. The data in Table 2 represent the fruits and vegetables most often recorded within a given country and therefore an estimate of the most consistent sources of dietary carotenoids for each group. The rank order of both dietary and breast milk carotenoids were unique for each of the nine countries. Although Philippine women consumed a similar number of total servings of fruits and vegetables as women in other countries, their predominant dietary fruit was banana, which is not high in carotenoid content. Mexican women reported the lowest number of total servings of fruits and vegetables (data not presented).

Breast milk carotenoid concentrations

Concentrations of breast milk carotenoids varied greatly among the nine countries sampled with the largest disparities in β-cryptoxanthin concentrations (8.8-fold) and smallest in lycopene and α-carotene concentrations (2.8- and 2.7-fold respectively) (Table 3). Total breast milk carotenoid concentrations varied 3.1-fold across countries and were highest in Japanese mothers. An earlier study by one of us (LC) demonstrated there is less sample-to-sample variation when fat-soluble nutrients are expressed relative to total milk lipid [7, 8]; therefore, carotenoid concentrations are presented as nmol/g lipid. Data are also presented in molar concentrations to allow comparison to similar carotenoid data in the literature.

As shown in Table 3, compared to the U. S. mean, Japan and Mexico had significantly higher breast milk β-cryptoxanthin and Chile, China, and Japan had significantly higher lutein/zeaxanthin. Concentrations of breast milk α-carotene were significantly higher than the U. S. mean only in Japanese women ($p \leq 0.05$, Dunnett's test). In contrast, breast milk β-carotene concentrations were significantly lower than U. S. concentrations only in Philippine mothers and lycopene concentrations were significantly less only in Chinese and Philippine mothers. Concentrations of pro-vitamin A carotenoids relative to non-provitamin A carotenoids ranged from 1.15 in Chile to 2.1 in Australia. There was no relationship between these ratios and retinol concentrations when expressed either as μmol/L or nmol/g lipid ($p \leq 0.05$).

Beyond concentration differences in carotenoids, the rank order of breast milk carotenoids was unique for each of the nine countries (Table 2). In breast milk samples from mothers in Australia, the United Kingdom, and the United States, β-carotene was the most abundant breast milk carotenoid, while in Canadian mothers, α-carotene concentrations were highest. The xanthophylls lutein/zeaxanthin, which are not retinol sources, were the most abundant carotenoids in breast milk of women from Chile, China, Japan, and the Philippines while in Mexico, β-cryptoxanthin was the breast milk carotenoid in highest concentration.

Total provitamin A carotenoids (β-carotene, α-carotene and β-cryptoxanthin) concentrations were strongly correlated with retinol in all countries except the Philippines (Table 4). Single carotenoids were less well correlated. The correlation was strongest and significant for β-carotene and retinol ($p \leq 0.05$) in all countries except Mexico and the Philippines. However, for α-carotene and retinol, correlations were significant only

Table 2 Milk carotenoids and dietary sources

Country	Dietary fruits/vegetables[1]	Dietary carotenoids, rank order[2]	Major milk carotenoids, rank order[3]
Australia	Apricot/Orange/Kiwi/Eggplant	β-carotene, lutein, β-cryptoxanthin, α-carotene	β-carotene, α-carotene, lycopene, lutein
Canada	Orange/Carrot/Banana/Apple	β-carotene, α-carotene, lutein, β-cryptoxanthin	α-carotene, β-carotene, lutein, lycopene
Chile	Potato/Tomato/Apple/Pumpkin	lycopene, β-carotene, α-carotene, lutein	lutein, β-carotene, α-carotene, lycopene
China	Orange/Peas/Asparagus/Carrot	β-carotene, α-carotene, lutein, β-cryptoxanthin	lutein, β-carotene, β-cryptoxanthin, α-carotene
Japan	Orange/Green Beans/Potato/Apple	lutein, β-carotene, β-cryptoxanthin, α-carotene	lutein, β-cryptoxanthin, β-carotene, α-carotene
Mexico	Papaya/Orange/Potato/Black Beans	β-cryptoxanthin, β-carotene, lutein	β-cryptoxanthin, β-carotene, lutein, α-carotene
Philippines	Banana/Green Beans/Potato/Melon	lutein, β-carotene, α-carotene	lutein, α-carotene, β-carotene, lycopene
United Kingdom	Orange/Banana/Potato/Apple	lutein, β-cryptoxanthin, β-carotene, α-carotene	β-carotene, lycopene, α-carotene, lutein
United States	Orange/Potato/Banana/Apple	lutein, β-cryptoxanthin, β-carotene, α-carotene	β-carotene, lutein, lycopene, α-carotene

[1] Foods most often reported in rank order, by 24-h intake record
[2] Carotenoid consumption from foods reported in rank order (32)
[3] Breast milk carotenoids, nmol/g lipid, ranked in order of concentration from highest to lowest

European Journal of Nutrition, Vol. 42, Number 3 (2003)

Table 3 Concentrations of major breastmilk carotenoids and retinol

Country	Total Carotenoids[1]	α-Carotene[1]	β-Carotene[1]	β-Cryptoxanthin[1]	Lutein/zeaxanthin[1]	Lycopene[1]	Retinol
			nmol/g lipid ± SEM (n)				
Australia	4.771 ± 0.338 (47)	0.865 ± 0.075 (48)	1.563 ± 0.177 (53)	0.623 ± 0.068 (52)	0.709 ± 0.049 (53)	0.813 ± 0.062 (52)	0.028 ± 0.001[3] (53)
Canada	4.184 ± 0.279 (53)	0.942 ± 0.082 (53)	0.916 ± 0.087 (55)	0.704 ± 0.077 (55)	0.795 ± 0.054 (55)	0.746 ± 0.057 (55)	0.030 ± 0.002[3] (55)
Chile	7.098 ± 0.898[2] (41)	1.002 ± 0.114 (41)	1.877 ± 0.248 (51)	0.639 ± 0.092 (51)	2.588 ± 0.334[2] (51)	0.882 ± 0.123 (50)	0.051 ± 0.005 (51)
China	4.885 ± 0.341 (46)	0.484 ± 0.048 (49)	1.240 ± 0.106 (52)	0.855 ± 0.079 (52)	2.016 ± 0.200[2] (52)	0.359 ± 0.034[3] (49)	0.025 ± 0.002[3] (52)
Japan	8.353 ± 0.501[2] (44)	1.337 ± 0.168[2] (46)	1.783 ± 0.138 (51)	2.331 ± 0.233[2] (51)	2.347 ± 0.152[2] (51)	0.700 ± 0.084 (48)	0.036 ± 0.002 (51)
Mexico	6.601 ± 0.437[2] (45)	1.002 ± 0.089 (47)	1.564 ± 0.147 (50)	1.620 ± 0.200[2] (49)	1.391 ± 0.095 (50)	0.970 ± 0.050 (49)	0.040 ± 0.002 (50)
Philippines	2.657 ± 0.285 (25)	0.896 ± 0.182 (35)	0.484 ± 0.044[3] (54)	0.265 ± 0.028 (55)	0.909 ± 0.107 (60)	0.362 ± 0.041[3] (45)	0.038 ± 0.003 (60)
United Kingdom	4.583 ± 0.283 (48)	0.937 ± 0.098 (49)	1.407 ± 0.135 (49)	0.515 ± 0.037 (50)	0.846 ± 0.098 (50)	0.996 ± 0.070 (50)	0.029 ± 0.001[3] (50)
United States	4.324 ± 0.356 (41)	0.629 ± 0.067 (41)	1.344 ± 0.115 (49)	0.454 ± 0.046 (49)	0.888 ± 0.096 (49)	0.770 ± 0.061 (49)	0.044 ± 0.003 (49)
			µmol/L ± SEM (n)				
Australia	0.185 ± 0.011[2] (47)	0.034 ± 0.003[2] (48)	0.060 ± 0.007[2] (53)	0.024 ± 0.002 (52)	0.027 ± 0.002 (53)	0.031 ± 0.002[2] (52)	1.086 ± 0.055 (53)
Canada	0.162 ± 0.009[2] (53)	0.036 ± 0.003[2] (53)	0.036 ± 0.003 (55)	0.027 ± 0.003 (55)	0.030 ± 0.001 (55)	0.030 ± 0.002[2] (55)	1.188 ± 0.066 (55)
Chile	0.170 ± 0.015[2] (41)	0.024 ± 0.002 (41)	0.044 ± 0.004 (51)	0.016 ± 0.002 (51)	0.057 ± 0.005[2] (51)	0.021 ± 0.002 (50)	1.242 ± 0.085 (51)
China	0.196 ± 0.014[2] (46)	0.019 ± 0.002 (49)	0.048 ± 0.004 (52)	0.035 ± 0.004[2] (52)	0.076 ± 0.008[2] (52)	0.014 ± 0.001 (49)	1.043 ± 0.088 (52)
Japan	0.293 ± 0.015[2] (44)	0.045 ± 0.004[2] (46)	0.062 ± 0.005[2] (51)	0.080 ± 0.008[2] (51)	0.077 ± 0.004[2] (51)	0.023 ± 0.002 (48)	1.230 ± 0.063 (51)
Mexico	0.223 ± 0.016[2] (45)	0.031 ± 0.002[2] (47)	0.051 ± 0.005 (50)	0.057 ± 0.008[2] (49)	0.044 ± 0.003[2] (50)	0.032 ± 0.002[2] (49)	1.321 ± 0.087 (50)
Philippines	0.127 ± 0.010 (25)	0.041 ± 0.010[2] (35)	0.022 ± 0.002 (54)	0.012 ± 0.001 (55)	0.035 ± 0.003 (60)	0.016 ± 0.002 (45)	1.624 ± 0.094[2] (60)
United Kingdom	0.159 ± 0.007 (48)	0.031 ± 0.003[2] (49)	0.048 ± 0.003 (49)	0.019 ± 0.002 (50)	0.027 ± 0.002 (50)	0.034 ± 0.002[2] (50)	1.052 ± 0.050 (50)
United States	0.114 ± 0.009 (41)	0.016 ± 0.002 (41)	0.037 ± 0.004 (49)	0.012 ± 0.001 (49)	0.026 ± 0.001 (49)	0.022 ± 0.002 (49)	1.227 ± 0.087 (49)

[1] Limit of detection of carotenoids by HPLC was 0.0025 µmol/L
[2] Significantly greater than US mean ($p \leq 0.05$)
[3] Significantly less than US mean ($p \leq 0.05$)

Table 4 Correlations between retinol and provitamin A carotenoids[1]

Country	α-Carotene	β-Carotene	β-Cryptoxanthin	Total provitamin A carotenoids
	Milk carotenoid concentrations nmol/g lipid			
Australia	0.47, 48, (0.0008)	0.59, 53, (0.0001)	0.35, 52, (0.0113)	0.64, 47, (0.0001)
Canada	0.19, 53, (0.1790)	0.38, 55, (0.0041)	0.10, 55, (0.4527)	0.29, 53, (0.0385)
Chile	0.56, 41, (0.0001)	0.78, 51, (0.0001)	0.65, 51, (0.0001)	0.74, 41, (0.0001)
China	0.23, 49, (0.1171)	0.31, 52, (0.0253)	0.11, 52, (0.4284)	0.36, 49, (0.0122)
Japan	0.15, 46, (0.3313)	0.50, 51, (0.0002)	0.29, 51, (0.0364)	0.49, 46, (0.0443)
Mexico	0.09, 47, (0.5373)	0.26, 50, (0.0705)	0.28, 49, (0.0558)	0.30, 46, (0.0443)
Philippines	–0.04, 35, (0.8374)	0.00, 54, (0.9955)	0.21, 55, (0.1191)	0.05, 32, (0.7945)
United Kingdom	0.28, 49, (0.0504)	0.48, 49, (0.0005)	0.31, 50, (0.0261)	0.48, 48, (0.0005)
United States	0.51, 41, (0.0007)	0.47, 49, (0.0006)	0.28, 49, (0.0549)	0.48, 41, (0.0014)
All countries combined	0.24, 409, (0.0001)	0.47, 464, (0.0001)	0.18, 464, (0.0001)	0.45, 403, (0.001)

[1] Correlation coefficient, number of paired values, and (probability of random occurrence). Correlation is significant at $p \leq 0.05$

for Australia, Chile, the United Kingdom and the United States. Similarly, β-cryptoxanthin and retinol concentrations were significantly correlated only in breast milk from Australia, Chile, Japan, and the United Kingdom, although correlations approached significance in the United States and Mexico. There was substantially less variation in breast milk retinol than in carotenoids among the nine countries sampled (2.0-fold, Table 3) and this is consistent with other studies [23].

Discussion

Consistent with previous work by one of us (LC) [8], provitamin A carotenoids as a group (α-carotene, β-carotene, and β-cryptoxanthin) accounted for more than half of the major carotenoids found in human milk (62 % previously in U. S. mothers [8]; 59 % this paper) in all the countries surveyed. These findings dispel earlier concerns that the non-provitamin A carotenoids, lutein/zeaxanthin and lycopene, comprise the majority of milk carotenoids [24].

It has been suggested [23, 25] that breast milk retinol concentration may be a useful indicator of vitamin A status of women and infants. In the present study, milk β-carotene concentrations were significantly correlated to retinol concentrations in all but two countries. However, total milk carotenoid concentrations were more significantly correlated to retinol in all countries except the Philippines (Table 4) where milk carotenoid concentrations were lowest. Because of their stronger correlation to milk retinol compared to β-carotene, provitamin A carotenoids may provide the better indicator of vitamin A status. This is of particular significance to populations such as Canada, Chile, China, Japan, Mexico, and the Philippines where β-carotene is not the predominant carotenoid in breast milk (Table 3).

Carotenoid concentrations in Japanese samples were substantially higher than in other countries, with concentrations of β-cryptoxanthin and α-carotene accounting for most of the increase. The two most likely reasons for this difference are method of collection and diet. In fact, because of cultural constraints, Japanese samples were collected by manual expression rather than electrical pump as in other countries. However, manual expression is typically less effective than electric pump at removing hindmilk in which lipid is concentrated [19]; thus, we would have predicted that carotenoid concentrations would have been lower in the Japanese samples. However, the average lipid concentration in these samples was 35.98 nmol/L, which was mid-range (range 28.07–47.5) for all countries. Thus, the most likely explanation for the results is that the diets of these mothers contained more vegetables high in α-carotene and β-cryptoxanthin than those in other countries.

The rank order of carotenoids measured in breast milk of U. S. mothers (β-carotene, lutein/zeaxanthin, lycopene, α-carotene, and β-cryptoxanthin), agrees well with previous reports [7, 8, 11, 15, 26]; however it is quite different from the rank order in other countries (Table 2). In healthy mothers, changes in breast milk carotenoids paralleled those in serum [8, 11]. These previous studies identified maternal diet as the variable of greatest influence to serum carotenoids [27, 28] and the same relationship appears to exist between maternal diet and breast milk carotenoids. The women participating in this study were apparently healthy and well nourished; however diets from their different regions varied greatly according to the availability of fruits and vegetables, personal preferences, and method of preparation. Although the objectives of this study did not include quantifying dietary carotenoid consumption, we did obtain a dietary recall to evaluate the general quality of the mother's diet. When the most frequently con-

sumed fruits and vegetables per site are compared to the mean values for major milk carotenoids for that country (Table 2), the rank order is sufficiently similar to suggest an association between dietary and breast milk carotenoids. Among other factors, the wide variation in bioavailability of carotenoids in foods complicates analysis of dietary carotenoid intake. Such factors include method of preparation of food, e. g., cooked versus raw, amount of fat and fiber (pectins) in the diet, and interactions of other nutrients, particularly iron, and zinc. [29, 30]. Whereas the efficiency of absorption for dietary vitamin A (retinol) is between 80 and 90 %, carotenoid absorption is slower and varies by carotenoid: lutein is more efficiently absorbed than β-carotene, the absorption of β-carotene can be as low as 40 % from certain foods [31].

Conclusions

Patterns of breast milk carotenoids varied greatly among countries in our study. Of the five major dietary carotenoids, total provitamin A carotenoids as a group were a better predictor of milk retinol than β-carotene alone. Concentrations of breast milk carotenoids differed substantially by country even where mothers were similarly well nourished and fruits and vegetables were plentiful. We conclude that carotenoid composition of breast milk is only useful for comparison to group means within that region. These results should be considered in developing international dietary recommendations for vitamin A and carotenoids for lactating women and nursing infants.

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